Differentiation of 1- and 2-Monoglycerides by Near-Infrared Absorption Spectroscopy[1]

H. SUSI, S.G. MORRIS, and W.E. SCOTT, Eastern Regional Research Laboratory,[2] Philadelphia, Pennsylvania

1- and 2-monoglycerides can be differentiated and mixtures analyzed by investigating the overtone of the OH stretching vibration, which occurs in the near-infrared spectral region close to 1.4 microns. The results are compared with data obtainable in the fundamental rock-salt region.

INFRARED spectroscopy of dilute solutions (1) and of crystalline films (2) has been shown to be of great value in the analysis and structure determination of mono-, di-, and triglycerides. One of the more difficult problems in glyceride analysis is differentiation and characterization of 1-monoglycerides and 2-monoglycerides. Chemical methods are tedious and time-consuming. Solution spectra obtained with infrared instruments equipped with conventional rock-salt optics differ in the 9-micron "fingerprint region," but the differences are relatively small and occur in a portion of the spectrum where many overlapping absorption bands are observed. Greater differences are observed if spectra of the solid compounds are obtained (2). Different thermal treatment of the 1-monoglycerides gives rise to different spectra, corresponding with the polymorphic forms, whereas 2-monoglycerides exist in only one form (2,3). The spectra of the most stable forms (β_L) differ considerably (2). However these procedures require great care. The solid state spectra moreover are not easily and directly utilizable for quantitative analytical determinations.

The purpose of this investigation was to establish the usefulness of near-infrared spectroscopy for differentiating between the two isomers and for the analysis of mixtures of 1- and 2-monoglycerides (in the absence of di- and triglycerides). Instruments with excellent photometric reproducibility and spectral resolution are now available for analytical spectrophotometry in the near-infrared spectral region. The main difference between the two isomers (OH groups in different intramolecular environment) appears to be of the kind which is well suited to investigation with near-infrared spectrophotometry where the greatest emphasis is on chemical groups and bonds involving hydrogen atoms.

Experimental

The 1-monoglycerides were prepared by the acylation of 1,2-acetone glycerol, followed by treatment with cold concentrated hydrochloric acid in ethyl ether; the 2-monoglycerides by the acylation of 1,3-benzylidene glycerol, followed by boric acid cleavage of the benzylidene-2-acylglycerol in dioxane.

The acetone glycerol was prepared according to Malkin and Shurbagy (4) except that the final distillation was over magnesium oxide instead of silver oxide. The 1,3-benzylidene-glycerol preparation was essentially that of Hibbert and Carter (5). The acylations were made with fatty acid chlorides in the presence of pyridine and chloroform solvents. To obtain the 2-monoglycerides from 1,3-benzylidene-2-acylglycerol, Martin's (6) method was used except that the alternate solvent, dioxane, was substituted for triethylborate.

Survey spectra in the conventional 2-15 micron region were obtained with a Perkin-Elmer Model 21 instrument equipped with rock-salt optics. Near-infrared absorption spectra from 1.0 to 2.5 microns were measured with a Cary Model 14 spectrophotometer. Cells of 1-cm. and 5-cm. path length were used. Sample concentrations ranged from 5 to 70 g./liter. Chloroform (Spectro-quality reagent, Matheson, Coleman, and Bell) was used as a solvent and as solvent compensator in the reference beam. The solvent was dried before use by bubbling through it a a stream of dry nitrogen. This method has proven highly successful in removing traces of H_2O from carbon tetrachloride. Solutions were prepared, and the absorption cells were filled in an atmosphere of dry nitrogen. Despite these precautions, small disturbances caused by H_2O absorption were observed. Fortunately these were found to be too small to interfere with the analytical measurements. Carbon tetrachloride is generally easier to obtain in a sufficiently anhydrous condition and is for this reason (among others, fewer absorption bands) usually preferred as a solvent for near-infrared work. Monoglycerides are unfortunately not sufficiently soluble in carbon tetrachloride; therefore chloroform was chosen for the present investigation.

Results and Discussion

Figure 1 shows the infrared spectra of 1-monostearin and 2-monostearin from 8 to 10 microns, *i.e.*, in the region where greatest differences are observed with conventional infrared equipment. The 2-isomer is seen to exhibit an additional weak band at 9.15 microns and a shift of the 9.5 micron band to a longer wavelength. These differences could be used for a rough qualitative examination, but they appear too small and occur in a region where too many bands overlap to be of great analytical value. Other pairs of 1- and 2-isomers showed quite similar behavior.

Survey spectra in the near-infrared spectral region were measured from 1.0 to 2.5 microns. Figure 2 shows the typical pair of 1-monopalmitin and 2-monopalmitin. The regions where strong chloroform absorption makes the instrument inexact or insensitive are marked with a dashed line. All bands with appreciable intensity can be associated with OH or CH bonds by comparison with previous near-infrared work (7). The first overtone of the OH stretching vibration is easily recognizable close to 1.4 microns. Although the two curves appear very similar at superficial examination, a closer study reveals measurable and reproducible differences in the 1.4 micron band. Figure 3 shows this band for 1-monopalmitin and 2-monopalmitin as obtained under optimum instru-

[1] Presented at the 53rd fall meeting, American Oil Chemists' Society, New York, October 17–19, 1960.

[2] Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

mental conditions. The largest difference between the two absorption curves is seen to occur close to 1.43 microns. The difference in absorptivity around 1.43 microns is actually smaller than at some wavelengths in the 8- to 10-micron region (Figure 1), but the higher instrumental accuracy, higher permissible path lengths, and more stable base lines observed with near-infrared instrumentation make the 1.43 micron band much more suitable for analytical applications. Figure 4 shows the dependence of absorbance on concentration for 1-monoglycerides and 2-monoglycerides at 1.430 microns. All absorbance values were measured relative to an A_0 determined by base-line points at 1.350 and 1.500 microns (*cf.* Figure 3). The straight lines obtained suggest that external hydrogen bonding does not interfere with the measurements within the covered concentration range. The absorbances of all investigated 1-monoglycerides and all investigated 2-monoglycerides fall on the same straight lines, indicating that absorption at 1.430 microns is not, or only very slightly, influenced by the length or degree of saturation of the hydrocarbon chains. Random scattering of the experimental points is somewhat larger than would be desirable for exact quantitative investigations. The random scattering results, to a

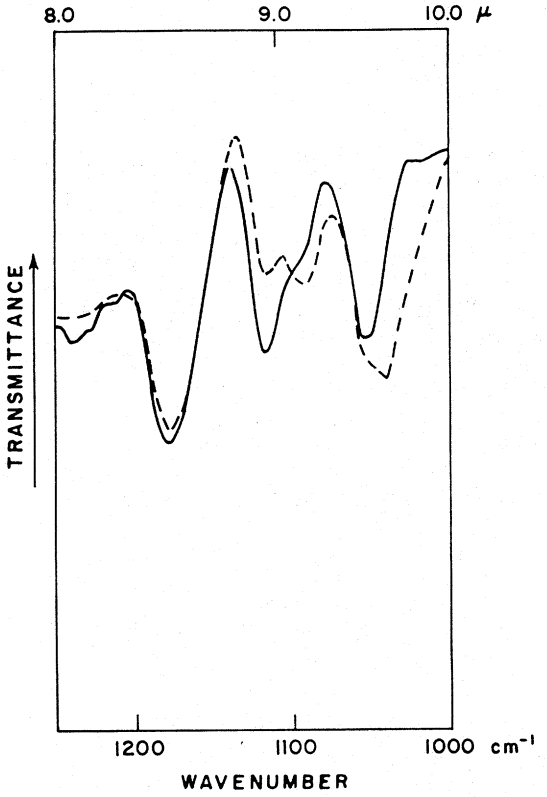

FIG. 1. Infrared spectra of 1-monostearin (solid line) and 2-monostearin (dotted line) from 8 to 10 microns. 18 g./liter in chloroform *vs.* chloroform; 0.2-mm. cells.

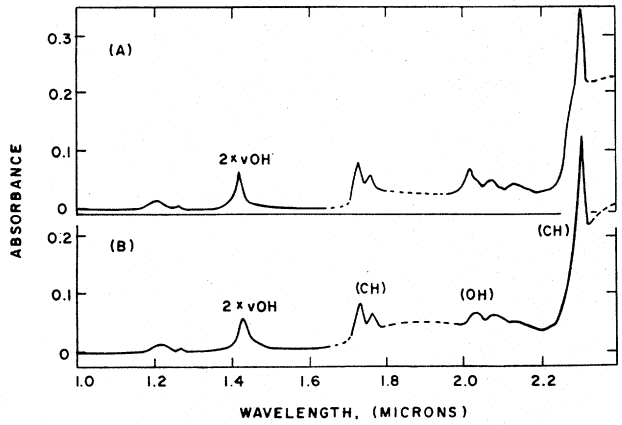

FIG. 2. Near-infrared survey spectra of 2-monopalmitin (A) and 1-monopalmitin (B). 8 g./liter in $CHCl_3$. --- regions where very strong $CHCl_3$ absorption interferes with measurements.

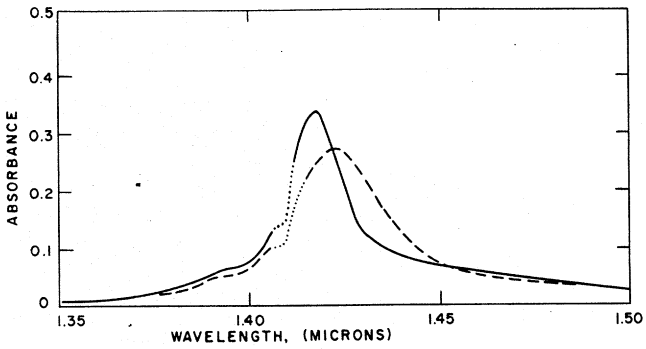

FIG. 3. First overtone of OH stretching band of 2-monopalmitin (solid line) and 1-monopalmitin (dashed line). Finely dotted line indicates interference by H_2O. 40 g./liter in 5-cm. cell.

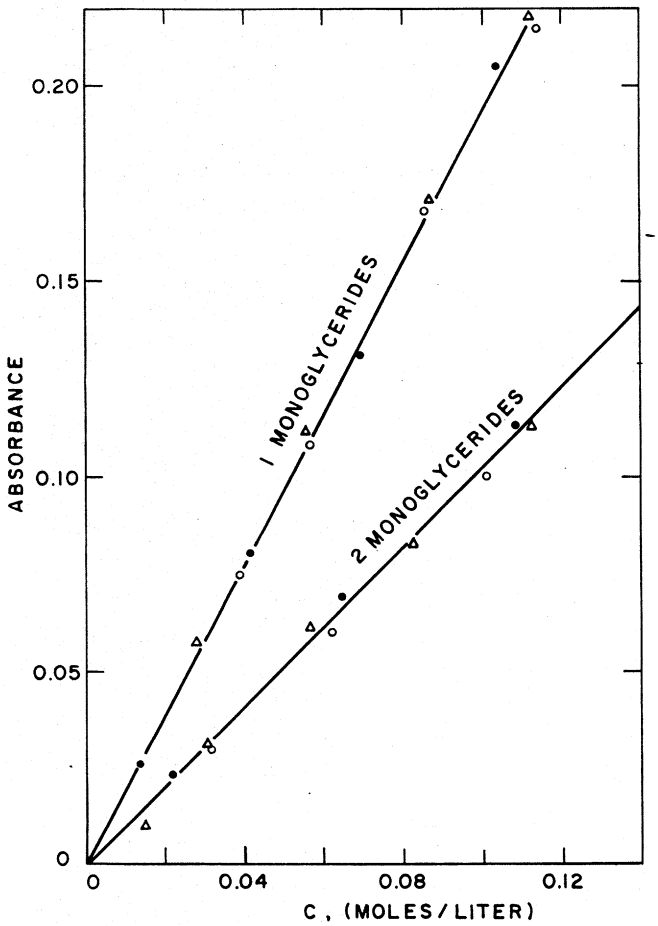

FIG. 4. Absorbance of 1-monoglycerides and 2-monoglycerides at 1.430 microns as a function of concentration. Open circles—monopalmitins; closed circles—monostearins; triangles—monooleins.

large degree, from a comparatively low signal-to-noise ratio, caused by the necessity to use dilute solutions in order to avoid external hydrogen bonding.

The absorbance of a mixture of 1- and 2-monoglycerides at a given wave-length is given by the relationship:

$$(1)\quad A = (c_1k_1 + c_2k_2)b$$
$$(2)\quad c = c_1 + c_2$$

A is the total absorbance; c_1 and c_2 are the molar concentrations of 1-monoglycerides and 2-monoglycerides; k_1 and k_2 are the molar absorptivities of 1-monoglycerides and 2-monoglycerides; b is the path length in cm. Rearrangement and substitution yields:

$$(3)\quad \%\ \text{monoglyceride} = (c_1/c)\ 100 = (A - ck_2\,b)\ 100/(k_1 - k_2)\ bc$$

Figure 4 leads to $k_1 = 0.39$ and $k_2 = 0.20$. The path length, b, was 5.0 cm.

Table 1 presents composition data calculated by equation (3) for mixtures of 1- and 2-monopalmitins and 1- and 2-monostearins of known composition. The agreement between the known composition and the spectroscopically measured composition is seen to be within a few per cent. This represents good agreement, considering the small absorbance differences between the two classes of compounds. The computed data are more exact than could be expected from measurements in the fundamental infrared region and approach the accuracy attainable in the ultraviolet region on samples with comparable differences in absorbance. While the procedure is not suitable for trace analysis, it constitutes a rapid method for rather accurate estimation of the abundance of position isomers in mixtures of 1- and 2-monoglycerides.

TABLE I

Known and Computed Composition of Mixtures of 1- and 2-Monoglycerides

Glyceride mixture	Total concentration (moles/liter)	Known amount of 1-monoglyceride (%)	Amount of 1-monoglyceride by eq. (3) (%)
1- and 2-monostearin.....	0.1093	26.8	23.2
	0.1073	47.8	48.1
	0.1052	69.8	73.0
1- and 2-monopalmitin.....	0.1110	16.4	18.0
	0.1127	40.3	42.0
	0.1139	57.6	57.2
	0.1154	78.9	79.6

Acknowledgment

The authors wish to express their gratitude to Sherman S. Pazner, who performed many of the spectroscopic measurements.

REFERENCES

1. O'Connor, R.T., DuPre, E.F., and Feuge, R.O., J. Am. Oil Chemists' Soc., *32*, 88 (1955).
2. Chapman, Dennis, J. Am. Oil Chemists' Soc., *37*, 73 (1960).
3. Daubert, B.F., and Clarke, T.H., Oil & Soap, *22*, 113 (1945).
4. Malkin, T., and el Shurbagy, M.R., J. Chem. Soc., 1628 (1936).
5. Hibbert, H., and Carter, N.M., J. Am. Chem. Soc., *51*, 1601 (1929).
6. Martin, J.B., J. Am. Chem. Soc., *75*, 5482 (1953).
7. Kaye, Wilbur, Spectrochim. Acta, *6*, 257 (1954).

[Received November 28, 1960]